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A MULTIPLE DOSE-ROUTE PHYSIOLOGICAL PHARMACOKINETIC
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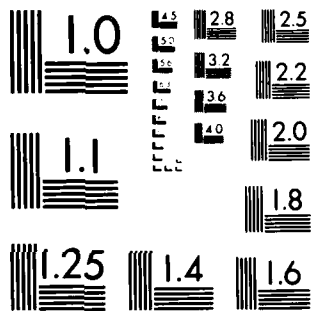
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A multiple dose-route physiological pharmacokinetic model for volatile chemicals using ACSL/PC

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ABSTRACT

The uptake, distribution, metabolism, and elimination of a chemical in an animal organism can readily be modeled in terms of a system of ordinary differential equations describing the mass balance for the chemical in each of the relevant tissues in the organism. Traditionally, pharmacologists and toxicologists have used greatly simplified models involving one or more empirically defined compartments in order to permit the generation of analytical solutions for the system of equations. These simplifications, however, entail a concomitant loss of predictive power, particularly in the case where non-linear biological processes cannot be properly incorporated into the model. With the advent of continuous simulation languages, the numerical solution of systems of differential equations can now be obtained quite easily, and the ability to generate an analytical solution should no longer be an important consideration in model definition. An example of a multi-purpose physiological pharmacokinetic model for volatile chemicals is presented. This model was written using ACSL/PC and demonstrates the ability of a physiologically-based description to accurately predict a wide variety of animal and human exposure scenarios, as well as the ease with which the model can be modified to incorporate additional features or to change the system description. By defining procedures in prologue files it was possible to modify the run-time environment under ACSL/PC in order to improve the ease of use of the model by non-technical personnel. Performance benchmarks for ACSL/PC operation on several personal computer systems are also presented.

INTRODUCTION

Traditional toxicology is essentially a descriptive discipline, focusing on the identification of toxic agents and the elucidation of the nature and mechanism of their toxicity. Of course there are also important quantitative aspects of toxicology, particularly in establishing a correspondence between the level of exposure to a toxic material and the likelihood of its producing a particular toxic effect. This correspondence is generally presented as a dose-response curve which can then be used to derive standard measures of toxicity such as the LD50 (lethal dose in 50% of test animals). Even these quantitative aspects, however, are primarily descriptive, with relationships derived empirically. Typically, curves are fitted to the data assuming a simple functional dependence -- such as linear, logarithmic, or log-normal -- and the suitability of a particular functional form is determined more by a posteriori measures of goodness of fit than by a priori theoretical considerations.

The descriptive character of toxicology is also reflected in the kinds of pharmacokinetic models that are usually employed in toxicological studies. A pharmacokinetic model is a mathematical description of the uptake, distribution, metabolism, and excretion of a chemical in a living system. Classical pharmacokinetic models are of two types: compartmental and noncompartmental. In the compartmental approach, the experimental system is represented by a collection of boxes and arrows. Each box, or compartment, reflects the total quantity of a particular chemical in a defined form or location, and the arrows represent the fluxes to and from each compartment. Mass balance equations are then written for the chemical in each compartment, and the solution of this system of differential equations describes the time course of the chemical in the animal system. In practice, due to the perceived complexity of biological systems and the lure of mathematical expediency, toxicologists and pharmacologists regularly use extremely simple compartmental descriptions in which the compartments possess little or no physiological correspondence. For example, many pharmacokinetic models are formulated in terms of a "central" compartment and a "peripheral" compartment without any clear definition of their precise physiological location or extent.

Historically, many simplifying assumptions were often necessary in order to obtain a tractable closed form solution of a compartmental model. The assumption that all processes in the biological system are linear, for example, leads to an exact solution in the form of a sum of damped exponential terms. In spite of the fact that numerical integration now makes possible the accurate solution of systems of arbitrary complexity, the traditional simplified models are still routinely used. The danger with these simple models lies in the extent to which they are formulated solely on the basis of conformance to data rather than physiological realism. That is, in common applications compartmental models are merely functional models, relating input and output variables on the basis of empirical evidence, rather than structural models in which an attempt is made to maintain a level of isomorphism with the animal system. As a result, the predictive power of traditional compartmental models is restricted to the range of conditions for which experimental observations exist. The other general class of pharmacokinetic models, the noncompartmental approach, is also essentially empirical, being based on the evaluation of a convolution integral, e.g.:

$$Q(t) = \int_0^t R_a(\tau) h(t - \tau) d\tau$$

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in which $Q(t)$ represents the total quantity of chemical in the system at time t , R_a is the rate of appearance of the chemical, and h is the impulse response of the system (determined, for example, from an intravenous injection).

In contrast to the empirically oriented models traditionally used by toxicologists and pharmacologists, there has been in recent years a growing use of pharmacokinetic models in which the actual physiology of the animal serves as the basis of a compartmental description. Although the principle of basing a pharmacokinetic description on physiological considerations was elaborated by Teorell in the 1930's [1], it was not until the 1970's that physiologically-based pharmacokinetic models began to appear in any numbers [2]. Two factors seem to have been important in the recent advancements in physiological pharmacokinetic modeling. The first was the availability of practical numerical integration algorithms and the computer hardware capable of performing them. The second was the application of these numerical techniques to problems in toxicology and pharmacology by investigators from more quantitatively oriented disciplines, principally chemical engineering [3]. Indeed, in spite of the greater predictive power of physiologically-based pharmacokinetic models, the application of these techniques has been limited by the general reluctance of the toxicology and pharmacology communities to undertake the more demanding mathematical and computational requirements of structural models. Modern continuous simulation languages offer the potential of greatly simplifying the development and application of physiologically-based pharmacokinetic models, and thus may help to overcome this obstacle. One of these, the Advanced Continuous Simulation Language (ACSL) [4], is now available for personal computers as well as larger systems. This paper describes a general physiologically-based pharmacokinetic model for volatile chemicals which takes advantage of the features of ACSL/PC to create a more friendly environment for its use by investigators with limited previous experience.

PHYSIOLOGICALLY-BASED PHARMACOKINETIC MODEL

A diagram of the model is shown in Fig. 1. The structure of the model reflects its intended use for volatile chemicals. For these chemicals, the principle route of excretion is via the lungs, with very little in urine or feces, so the latter two compartments can be eliminated. The various tissues and organs of the animal system can be aggregated into four lumped compartments: fat, muscle, richly perfused tissues (such as brain and kidney), and the liver (the preponderant site of metabolism). This particular model tracks only the parent chemical itself, rather than metabolites, so metabolism is considered only as a means of elimination of the parent chemical. The arrows in the diagram trace the flux of chemical between compartments in the blood or alveolar air, and the structure shown is representative of the standard mammalian architecture. The model described here is a generalization of a model originally developed for styrene [5] which successfully described the inhalation pharmacokinetics of styrene in both rats and humans. This ability to perform a reliable scale-up from experimental animals to humans is the basis for an important application of physiologically-based pharmacokinetic models in chemical risk assessments.

In the following description of the model, the equations will be presented in the form they are typed into the ACSL source code, for ease of comparison with the tables. The input parameters required by the model are defined in Table 1.

Table 1. Model Input Parameters

Physiological:

QPC	Alveolar Ventilation (L/hr - 1 kg animal)
QCC	Cardiac Output (L/hr - 1 kg animal)
QLC	Liver Blood Flow (fraction of cardiac output)
QFC	Fat Blood Flow (fraction of cardiac output)
BW	Body Weight (kg)
VLC	Liver Volume (fraction of body weight)
VFC	Fat Volume (fraction of body weight)

Chemical:

PL	Liver/Blood Partition Coefficient
PF	Fat/Blood Partition Coefficient
PS	Slowly Perfused Tissue/Blood Partition Coefficient
PR	Richly Perfused Tissue/Blood Partition Coefficient
PB	Blood/Air Partition Coefficient
MW	Molecular Weight (g/mol)

Biochemical:

VMAXC	Maximum Velocity of Metabolism (mg/hr - 1 kg animal)
KM	Michaelis-Menten Constant (mg/L)
KFC	First Order Rate Constant (/hr - 1 kg animal)

Exposure Conditions:

CONC	Concentration in Chamber (ppm)
PDOSE	Oral Dose (mg/kg)
KA	Oral Uptake Rate Constant (/hr)
IVDOSE	Intravenous Dose (mg/kg)
TCHNG	Time at End of Exposure Period (hrs)
TSTOP	Time at End of Experiment (hrs)

Physiological parameters are obtained from the literature, and chemical-specific partition coefficients can be determined by simple *in vitro* techniques [6]. Biochemical parameters can be determined by several methods, including a variation of the technique used for partition coefficients and an *in vivo* method requiring analysis with another physiologically-based pharmacokinetic model [7]. To facilitate animal scale-up, the model automatically adjusts the value of the physiological and biochemical parameters as a function of body weight based on accepted allometric relationships. All of the input parameters listed in Table 1 are declared as ACSL constants so that they can be changed interactively when the model is exercised. (The richly perfused tissue and muscle compartments are automatically adjusted to compensate for changes in the liver and fat compartment parameters, respectively.) The output variables are defined in Table 2. Any of these are available for interactive plotting after the model is run.

Table 2. Model Output Variables

T	Time (hrs)
CA	Arterial Blood Concentration (mg/l)
CV	Venous Blood Concentration (mg/l)
CXPPM	Exhaled Air Concentration (ppm)
CP	Chamber Concentration (ppm)
CF	Concentration in Fat (mg/l)
CVF	Concentration in Venous Blood Leaving the Fat (mg/l)
CL	Concentration in the Liver (mg/l)
CVL	Concentration in Venous Blood Leaving the Liver (mg/l)
CS,CVS	Slowly Perfused Tissues/Venous Blood Concentrations (mg/l)
CR,CVR	Richly Perfused Tissues/Venous Blood Concentrations (mg/l)
RAM	Instantaneous Rate of Metabolism (mg/hr)
AM	Total Amount Metabolized (mg)
RAI	Rate of Input from Stomach (mg/hr)
MR	Amount Remaining in Stomach (mg)
AX	Total Amount Exhaled (mg)
AUCB	Area Under the Curve in the Blood (mg*hr/l)

The mass-balance for each of the four compartments is described very simply using the ACSL integration macro. The most complicated compartment, the liver, is described as follows:

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RAL = QL*(CA-CVL) - RAM + RAO
RAM = (VMAX*CVL)/(KM+CVL) + KF*CVL*VL
RAO = KA*MR
AL = INTEG(RAL,0.)
CL = AL/VL
CVL = CL/PL
AUCB = INTEG(CL,0.)

```

The first statement is the mass balance equation for the liver compartment, and states that the rate of change of the amount of chemical in the liver (RAM) depends on three factors. The first, the net exchange with the blood perfusing the liver, is defined as the blood flow rate through the liver (QL) times the difference in concentration between the arterial blood (CA) and the venous blood leaving the liver (CVL). This simple description results from the fact that for these volatile chemicals, diffusion limitations can safely be ignored and a flow-limited model is appropriate. The second term represents the rate of metabolism (RAM) and is defined in the next statement as consisting of a saturable (Michaelis-Menten) pathway and a first-order pathway, if both VMAX and KF are non-zero (VMAX and KF are the scaled values of VMAXC and KFC, respectively). It is worth noting that we can use RAM before it is defined because of the handy feature that ACSL automatically reorders the statements to avoid any undefined variables. The last term (RAO) is included to incorporate oral dosing. Physiologically, the blood perfusing the stomach and intestines flows directly to the liver. Thus in the model, any chemical introduced orally is shown to appear as a direct input to the liver. The specific functional form, first-order in the amount remaining in the stomach (MR), is consistent with experimental results for uptake of oral doses given in an aqueous vehicle. Oral dosing in oil shows more complicated kinetics [5]. For dosing other than oral, MR is initialized to zero.

The fourth statement simply defines the amount of chemical in the liver (AL) to be one of the state variables defining the system. This state variable

is the integral of RAL, using an initial value of zero. The next two statements define the average concentration in the liver (CL) and in the venous blood leaving the liver (CVL). In this venous equilibration model it is assumed that these two concentrations are simply related by the tissue/blood partition coefficient. The final statement defines the area under the curve in the liver to be the integral of the liver concentration. The equations for the other three compartments are much simpler than the ones shown above since they do not contain the terms for metabolism or oral uptake. The venous blood from the various compartments joins to form the mixed venous blood:

$$CV = (QF*CVF + QL*CVL + QS*CVS + QR*CVR + IV)/QC$$

Here, IV is a function defined to be non-zero only for the case of an intravenous injection, and then approximates an impulse function. The arterial blood concentration (CA) is calculated assuming that steady state is continually achieved between alveolar air (CI) and pulmonary blood:

$$CA = (QC*CV + QP*CI)/(QC + (QP/PB))$$

During an inhalation exposure, CI represents the chamber concentration. At all other times it is zero, and the lungs serve as a route of elimination.

IMPLEMENTATION OF ACSL MODEL

The statements described above define the system of ordinary differential equations which must be solved to obtain the time-course of the chemical. In general, these physiologically-based models are stiff, that is the time constants for the various compartments differ by many orders of magnitude. Therefore, the backward differentiation method of Gear is routinely selected to perform the numerical integration. Comparisons with other algorithms available in ACSL show that, as expected, the Gear algorithm is consistently faster than the Adams method for these systems. Although the Runge Kutta methods can be made to run faster than the Gear algorithm, depending on the choice of step size, they are subject to instability and it is not possible to incorporate automatic per-step error tolerances. Therefore the Gear algorithm is preferred. Benchmarks for several hardware systems running this simple model are shown in Table 3.

Table 3. Model Performance Benchmarks

Computer System	Translation/ Compilation/ Linking (min)	Execution (min)
IBM-PC (floppy disk)	18	8
IBM-XT (10 Mb disk)	12	8
IBM-XT with math coprocessor	9	1.1
IBM-AT (20 Mb disk)	*	2.5
IBM-AT with math coprocessor	2.2	.6
CDC Cyber 170/845 (mainframe)	.5	.01

* Not determined

It can be seen that a math coprocessor chip is a necessary item for interactive modeling. Even then,

for some of the more complex models we have developed, execution times of five to twenty minutes are not uncommon. Therefore, some modeling is still better done in a mainframe environment. Nevertheless, for the model described here and many others, execution times with a math coprocessor are quite reasonable.

Some of the advantages of using a continuous simulation language, instead of writing a model in a standard programming language such as Fortran or Basic, should already be apparent. For one, the model is more readable due to the free form entry. It is also much easier to modify. Adding another compartment is simply a matter of typing in the statements that describe it. There is no need to modify the parameters in the call to the numerical integration subroutine or to provide additional input and output statements for the new parameters and variables -- this is all handled automatically. Interactive execution is more efficient since it does not rely on a sequence of queries from the executing program, but rather utilizes a run-time command interpreter. The friendliness of the run-time environment can be increased even further by the use of a prologue file that is invoked at the beginning of an interactive session. The prologue file for this model, for example, performs the following sequence of tasks:

1. Sets the default title that is to be printed above all plots.
2. Tells the simulation which variables are to be recorded for subsequent use in plots (those listed in Table 2), and which are to be output to the screen.
3. Defines new command words that represent a sequence of ACSL commands. For example, the commands IN, OR, and IV are defined by procedures that set the appropriate values of parameters for inhalation, oral, and intravenous exposures, respectively, and then run the model and plot the results in a standard format.
4. Turns control over to the keyboard for interactive processing.

The use of a prologue file thus makes it possible to hide the details of ACSL from those unfamiliar with the language, without restricting the more advanced user.

APPLICATION OF MODEL

To demonstrate the remarkable predictive power of physiologically-based pharmacokinetic models, the ACSL program described above was used to simulate a variety of exposures for which experimental data were available. The results of these simulations are presented in Figs. 2-5. These figures were actually drawn by ACSL/PC on a Hewlett-Packard plotter, and the data points were then added with another program. Fig. 2 shows the predictions of the model for the time course of dibromomethane in the venous blood of rats following intravenous injections of 65.5 mg/kg and 13.1 mg/kg. The points represent data collected in our laboratory using an exterior jugular vein cannula to obtain serial blood samples [8]. It is important to realize that the model was not adjusted to match these data. The physiological, chemical, and biochemical parameters had been previously determined [7] by the techniques

described above, and these are listed in the first column of Table 4. These parameters depend on the

Table 4. Parameter Values Used in Simulations

	Dibromomethane (in rats)	Methylene Chloride (in humans)
QPC	14.	14.
QCC	14.	14.
QLC	.25	.25
QFC	.09	.09
BW	.225	70.
VLC	.04	.04
VFC	.07	.20
PL	.932	.732
PF	10.7	6.18
PS	.547	.408
PR	.932	.732
PB	74.1	9.7
MW	173.85	84.9
VMAXC	12.5	4.
KM	.4	.4
KFC	.7	2.

physiology of rats and the properties of dibromomethane, not on the exposure scenario. Thus the only parameters which could be varied were the body weight and those describing exposure conditions. These, of course, are completely determined by the experiment to be simulated, so that there are no adjustable parameters. The agreement between model predictions and actual data depends upon the adequacy of the physiological description used.

The simulation of oral dosing is shown in Fig. 3. The data for 50 mg/kg and 10 mg/kg oral doses of dibromomethane were obtained in the same fashion as for the intravenous study [9]. In this case, however, one of the parameters needed for the model, the rate of uptake (KA), was not known. Actually measuring the rate of uptake of dibromomethane from the stomach of a rat is a difficult experimental procedure. Therefore, the value of KA used in Figure 2 was selected on the basis of matching the data. The estimated value, 10/hr, is similar to the 5.5/hr previously estimated for a different chemical, styrene [5].

In the experiments depicted in Fig. 4 the animals were exposed to a constant concentration (200 ppm or 100 ppm) of dibromomethane for 4 hrs, and were then removed from the exposure chamber. The concentration of dibromomethane in venous blood was determined during the post-exposure period in the same manner as for the other dosing methods [8]. In the simulation of the inhalation exposures all of the model parameters were known *a priori*. Thus, as in the case of intravenous injection, no parameter adjustment was possible. The ability of the model to predict the experimental results is due to its physiological basis.

As a final demonstration of the potential of a physiologically-based pharmacokinetic model, the same ACSL/PC program was used to predict the time-course of another chemical, methylene chloride, in a different species, humans. To accomplish this the parameter values were simply changed to those listed in the second column of Table 4. Except for the blood/air partition coefficient, which was measured for human blood, the chemical and biochemical

parameters are those which had previously been determined for rats [7]. All that was necessary to scale up the model from rat to human was to increase the body weight and the percentage of body fat. (Rats are leaner than people.) As mentioned before, the model automatically scales the physiological and biochemical parameters according to established allometric relationships. The predictions of the model using these parameter values are shown in Fig. 5 together with actual experimental data collected from human volunteers [10]. The data in this case are for 6-hr inhalation exposures to 350 ppm and 100 ppm of methylene chloride, and the agreement of the model with the data exemplifies the fact that for these volatile, lipophilic chemicals a very simple description of mammalian physiology is adequate to permit accurate species (as well as dose and route) extrapolation.

CONCLUSION

This paper has focused on the greater predictive capability of pharmacokinetic models possessing a physiological structure. In particular, the ability to extrapolate between different doses, routes, and species has been demonstrated. The ability to extend beyond the range of experimental data is not the only important advantage of a physiologically-based model, however. Perhaps of equal importance is its ability to provide a conceptual framework for understanding the complex interactions that control the distribution and elimination of a chemical in a biological system. Using a physiological model the investigator can describe his biological hypotheses in mathematical terms, make quantitative predictions based on his description, and revise his hypothesis in light of any discrepancy between the prediction and experiment. Thus the response to the failure of a physiological model to agree with experimental data is not to adjust some parameter to obtain a fit, but rather to consider how the physiological description underlying the model might be inadequate.

Once a model has been validated for a particular application it can also be used to perform quality control during data collection, determining whether or not a given data set is consistent with data from other experimental conditions. Of course, sometimes a disagreement might indicate a shortcoming in the model, but a re-examination of inconsistent data can often lead to the discovery of calculational or procedural errors which would have gone unnoticed if the data were collected uncritically. Another application of a validated model is to provide estimates of variables or parameters not accessible to direct experimental determination. The estimation of K_A for dibromomethane in this paper is one example. A less trivial example is the analysis of gas uptake experiments using a physiological model [7]. In this case, the nature of the *in vivo* metabolic processes in the livers of rats in a closed, recirculated chamber is deduced from the decrease in concentration of a chemical in the air of the chamber, without ever sampling the animal.

The development of continuous simulation languages for personal computers, such as ACSL/PC, has made possible the general use of physiologically-based pharmacokinetic models. However, the extent to which these methods will be used instead of the more restricted, but simpler, traditional techniques is as much a function of the

viewpoint of the investigator as it is of the ease of application. What is needed is a change of perspective from one that focuses on the analysis of experimental data to one that focuses on the description of the physiological/biochemical system under study.

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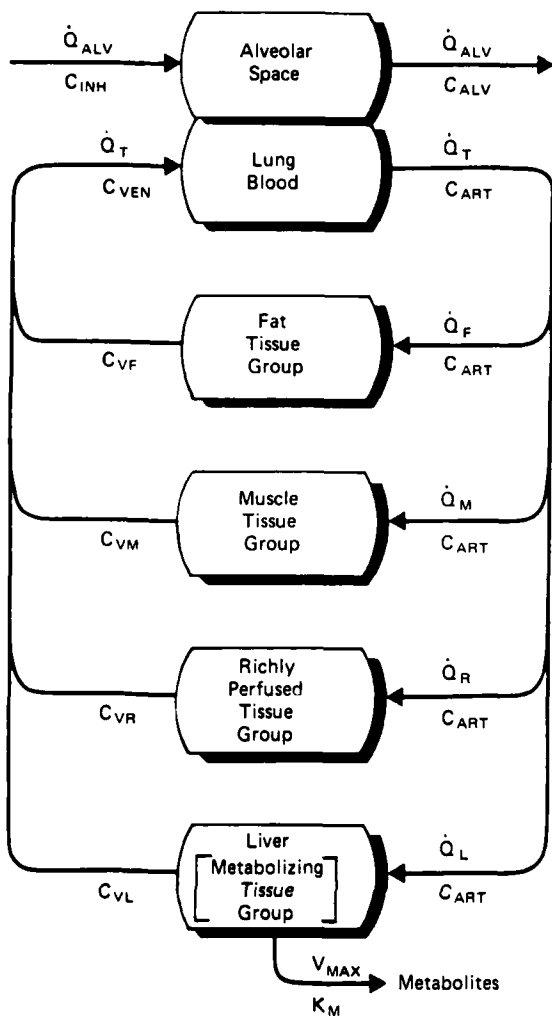


Fig. 1. Diagram of Physiologically-Based Pharmacokinetic Model.

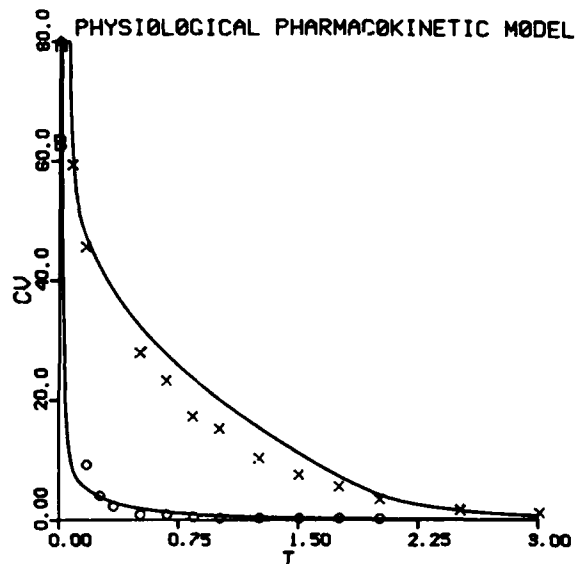


Fig. 2. Dibromomethane concentration in mixed venous blood of rats given intravenous injections of 65.5 mg/kg (top) and 13.1 mg/kg (bottom). Solid lines are the predictions of the model, and points represent the average of 2 (top) or 3 (bottom) animals [8].

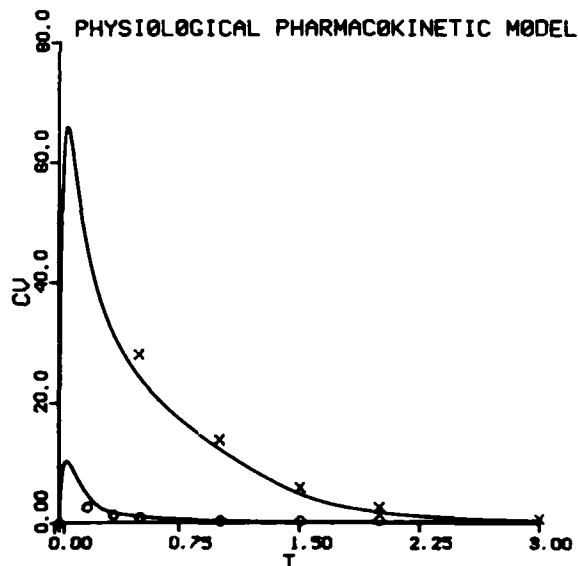


Fig. 3. Dibromomethane concentration in mixed venous blood of rats given oral doses of 50 mg/kg (top) and 10 mg/kg (bottom), administered as a saline solution. Solid lines are the predictions of the model assuming a first-order uptake from the stomach ($K_A = 10$), and points represent the average of 4 (top) to 2 (bottom) animals [9].

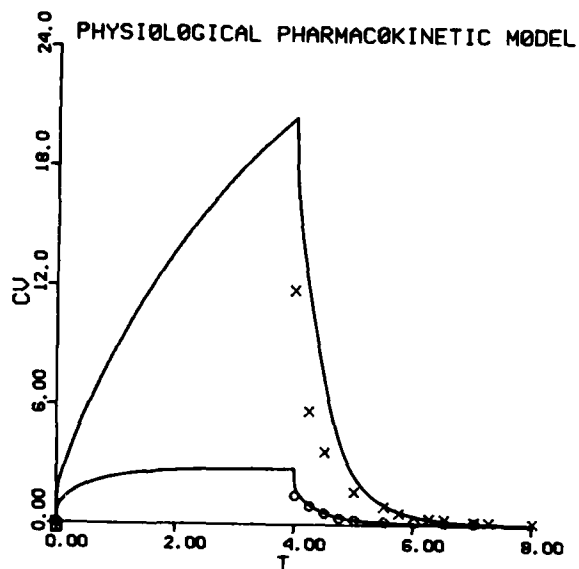


Fig. 4. Dibromomethane concentration in mixed venous blood of rats during and after 4-hr inhalation exposures at 200 ppm (top) and 100 ppm (bottom). Solid lines are the predictions of the model, and points represent the average of 3 animals [8].

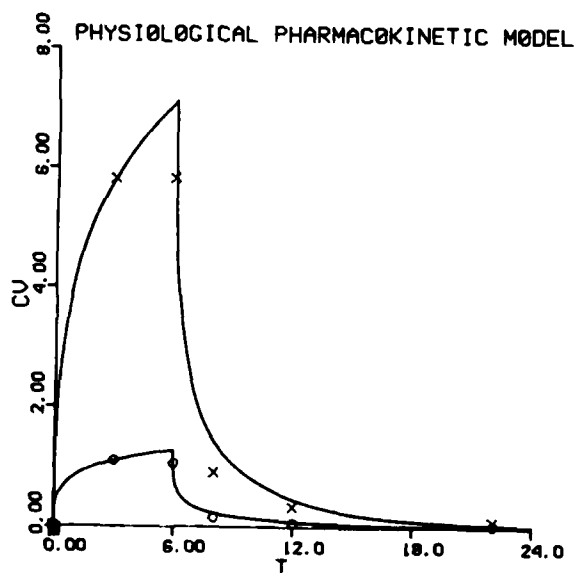


Fig. 5. Methylene chloride concentration in mixed venous blood of human volunteers during and after 6-hr inhalation exposures at 350 ppm (top) and 100 ppm (bottom). Solid lines are the predictions of the model, and points represent the average of 4 individuals.

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